REMARKS

Claims 1 and 53-77 are now pending. Claim 1 has been amended, and claims 53-77 have been added. Support for claims 53-74 is found in the original claims and in the Examples. Support for method claims 73-77 is found in original claims 10-11 and 22-23. It is respectfully requested that the Examiner examine these method claims in accordance with MPEP § 821.04. Applicants expressly reserve the right under 35 U.S.C. §121 to file a divisional application directed to the subject matter in cancelled claims 2-9, 24-29 and 42-52 during the pendency of this application, or an application claiming priority from this application.

Invention

The invention encompasses the production of starter and/or extender units for the production of polyketides in host cells that do not produce them and in host cells in which an enhanced production is beneficial. *E. coli* and *S. coelicolor* host cells have been exemplified in the application, and thus, the present claims are directed to these host cells to place the application in condition for prompt allowance without prejudice to the Applicants' right to present claims to subject matter deleted from the claims by this amendment in a continuation or divisional application. The propionyl CoA *(pcc)* expression system and the *mat* expression system are two of the expression systems set forth in the present claims as capable of making these starter and/or extender units.

Information Disclosure Statement

It is noted that the information disclosure statement filed on December 11, 2002, has been reviewed and initialed by the Examiner.

Objections to the Specification

The first paragraph of the application has been amended to reflect that the present application claims priority to the provisional applications listed.

The application has been amended to include the proper references to patent numbers as requested by the Office.

The title has been amended substantially in accordance with the title requested by the Office.

Therefore, the objections to the specification have been addressed, and the objections should be withdrawn.

Claim Objections

Claims 4-5, 8-9, and 27 to which the Office objects have been deleted or amended such that these objections are moot. The Applicants therefore request that the objections to the claims be withdrawn.

Claim Rejections - 35 U.S.C. §112

Claims 1-9, 24-29, and 42-43 are rejected under 35 U.S.C. 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the terms "polyketide" and "complete polyketide" stating that they are confusing. The latter term has been deleted from the claims, or the claims themselves have been deleted or amended, thus rendering this rejection as to the deleted claims moot. The term "polyketide" is respectfully submitted to be clear. In claims 1 and 69, the term "polyketide" refers to the compound made by a modular PKS, such as the polyketide defined in claims 60 and 74. The term "polyketide" in claim 61 refers to a compound, for instance, made by a

Streptomyces host cell containing the modular PKS defined in claim 64. The Applicants therefore respectfully request withdrawal of this rejection.

Claims 1-9 and 24-29 are rejected under 35 U.S.C. 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter with regard to the terms "starter unit" and "extender unit" stating that they are unclear in view of the broad and unclear definition of the term "polyketide." The former two terms have been deleted from the claims or the claims themselves have been deleted thus rendering this rejection moot. The Applicants therefore respectfully request withdrawal of this rejection.

Claims 1-9 and 24-29 are rejected under 35 U.S.C. §112, second paragraph, as being allegedly incomplete for omitting essential elements, such omission amounting to a gap between the elements. Applicants respectfully traverse this rejection, but to expedite prosecution of this application to allowance, claim 1 has been amended to include an expression system for a phosphopantetheinyl transferase. The Applicants therefore respectfully request withdrawal of this rejection.

Claim 9 is rejected under 35 U.S.C. §112, second paragraph, as being allegedly indefinite a) for failing to particularly point out and distinctly claim the subject matter, with regard to the term "said at least one polyketide synthase protein" as not having proper antecedent basis in its parent claims 1 or 4, and b) for failing to particularly point out and distinctly claim the subject matter, with regard to the term "derived from" is unclear. This claim has been deleted, rendering this rejection moot. The Applicants therefore respectfully request withdrawal of this rejection.

Claims 1-9, 24-29, and 42-43 are rejected under 35 U.S.C. §112, first paragraph, written description. Applicants traverse this rejection, but to expedite prosecution of the application to allowance, claim 1 has been amended, and claims 53-60 and 73-77 have been added such that

particular expression systems for pcc, phosphopantetheinyl transferase, and a modular PKS are defined therein. Claims 53-60 and 75-77 have been added, which depend from claim 1, of which claims 57-58 are directed to a lack of a functional endogenous pathway for propionate catabolism, such as the *prp*AD operon. As some catabolism of propionate would not prevent polyketide synthesis, and as substrates other than propionate may be converted by pcc (page 6, lines 23-24), lack of an expression system for catabolizing propionate was not included in the independent claims. Claims 61-74 have been added that are directed to the host cell containing the *mat* operon and an expression system for a modular PKS, and further, in claims 69-74, phosphopantetheinyl transferase. These claims do not define cells that rely on propionate as a starter unit. Applicants respectfully submit that these claims conform with the cited section.

Claims 1-9, 24-29, and 42-43 are rejected under 35 U.S.C. §112, first paragraph, enablement. Applicants gratefully acknowledge the Examiner's indication that the *mat*ABC genes, *pccB/accA2* genes and/or an inactivated *prp* operon have enabling support in the specification.

Claims 1, 53-60 and 75-77 are now directed in part to an *E. coli* host cell that contains a propionyl CoA carboxylase (pcc) expression system. The present application on page 7, line 19, includes *pccB* and *accA2* genes encoding pcc as well as homologs thereof. Thus, there is also sufficient enabling support for this expression system. The Applicants therefore respectfully request withdrawal of this rejection as applied to these claims.

Claim 61-74 relate to the *mat* operon. Independent claims 61 and 69 are each directed in part to the *mat*B gene. This gene is sufficient to make the thioester starter units as disclosed on page 6, lines 3-4, and on page 7, lines 2-4; thus, there is sufficient enabling support for cells

containing the *mat*B gene in this group of claims. The Applicants therefore respectfully request withdrawal of this rejection as applied to these claims.

Claims 58 is directed to an *E. coli* host cell that has no functional endogenous pathway for propionyl catabolism, and one particular embodiment of such a host, an *E. coli* cell with a disabled *prp* operon, respectively. The specification specifically describes an *E. coli* host cell that does not produce catabolizing enzymes on page 5, lines 21-22, and thus, it is respectfully submitted that these claims are of proper scope.

Therefore, it is respectfully submitted that this rejection is now moot with respect to claim 1 and that claims 53-77 are of proper scope.

Claim Rejections - 35 U.S.C. §102

The rejections under 35 U.S.C. §102(a) of claims 1-3 as being anticipated by the a) An, *et al.* and b) Rodriguez, *et al.*, references are traversed. Applicants acknowledge the An reference in Example 1 and the Rodriguez reference in Example 4 of the present specification. The host cells of claim 1, as amended, contain an expression system for a modular PKS, and therefore, it is respectfully submitted that the present amended and new claims distinguish over these references.

The rejections under 35 U.S.C. §102(b) of (a) claims 1-3 over Spratt, *et al.*; b) claims 1-3 and 8 over Quadri, *et al.* (IDS reference 13); (c) claims 1-2, 4, 6-7, 9, and 24-26 over Kao *et al.* (IDS reference 9); (d) claims 1-4, 9, and 24 over Stassi *et al.* (IDS reference 15); (e) claims 1, 2, 4, 9, and 24 over Tang *et al.* (IDS reference 16); and (f) claims 1-9, 24-29, and 42-43 over Barr *et al.* (IDS reference 2) are traversed. The claims have been amended or deleted such that all of these rejections are no longer at issue. It is respectfully submitted that new claims 53-84 are not anticipated by any of the cited references.

Withdrawal of these rejections therefore is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 286002021100.

Respectfully submitted,

Dated: August 7, 2002 By: _____

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Exhibit A - Version with Markings to Show Changes

In the Title

Please replace the title with the following title:

[BIOSYNTHESIS OF POLYKETIDE SYNTHASE SUBSTRATES] *E. COLI* AND *STREPTOMYCES* HOST CELLS THAT SYNTHESIZE METHYLMALONYL COA

In the Specification

Please replace the first paragraph on page 1 with the following paragraph:

This application [is related] claims priority under 35 U.S.C. § 119(e) to application Serial No. 60/159,090 filed 13 October 1999; Serial No. 60/206,082 filed 18 May 2000; and Serial No. 60/232,379 [(Atty. Docket 28600-30211.21)] filed 14 September 2000, which are expressly incorporated herein by reference.

Please replace the paragraph on page 2, commencing at line 21, with the following paragraph:

Additional problems that may need to be surmounted in effecting the production of polyketides in procaryotic hosts, especially those which do not natively produce polyketides, include the presence of enzymes which catabolize the required starter and/or extender units, such as the enzymes encoded by the *prp* operon of *E. coli*, which are responsible for catabolism of exogenous propionate as a carbon and energy source in this organism. In order to optimize production of a polyketide which utilizes propionyl CoA as a starter unit and/or utilizes its carboxylation product, methylmalonyl CoA as an extender unit, this operon should be disabled, except for that portion (the E locus) which encodes a propionyl CoA synthetase. Any additional loci which encode catabolizing enzymes for starter or extender units are also advantageously disabled.

Please replace the paragraph on page 4, commencing at line 11, with the following paragraph:

In the illustrative example below, *E. coli* is modified to effect the production of 6-dEB, the polyketide precursor of erythromycin. The three proteins required for this synthesis, DEBS1, DEBS2 and DEBS3 are known and the genes encoding them have been cloned and sequenced. However, a multiplicity of additional PKS genes have been cloned and sequenced as well, including those encoding enzymes which produce the polyketide precursors of avermectin, oleandomycin, epothilone, megalomycin, picromycin, FK506, FK520, rapamycin, tylosin, spinos[ad]yl, and many others. In addition, methods to modify native PKS genes so as to alter the nature of the polyketide produced have been described. Production of hybrid modular PKS proteins and synthesis systems is described and claimed in U.S. patent 5,962,290. Methods to modify PKS enzymes so as to permit efficient incorporation of diketides is described in U.S. patent 6,080,555. Methods to modify PKS enzymes by mixing and matching individual domains or groups of domains is described in U.S. Serial No. 09/073,538. Methods to alter the specificity of modules of modular PKS's to incorporate particular starter or extender units are described in [U.S. Serial No. 09/346,860, now allowed] U.S. Patent No. 6,221,641. Improved methods to prepare diketides for incorporation into polyketides is described in U.S. Serial No. 09/492,733. Methods to mediate the synthesis of the polyketide chain between modules are described in U.S. Serial No. 09/500,747. The contents of the foregoing patents and patent applications are incorporated herein by reference.

Please replace the paragraph on page 11, commencing at line 10, with the following paragraph:

For either *in vivo* or *in vitro* production of the polyketides, acyl transferase domains with desired specificities can be incorporated into the relevant PKS. Methods for assuring appropriate specificity of the AT domains is described in detail in [U.S. Patent Application 09/346,860 filed 2 July 1999] U.S. Patent No. 6,221,641, the contents of which are incorporated herein by reference, to describe how such domains of desired specificity can be created and employed. Also relevant to the use of these enzymes *in vitro* or the genes *in vivo* are methods to mediate

polyketide synthase module effectiveness by assuring appropriate transfer of the growing polyketide chain from one module to the next. Such methods are described in detail in U.S. Serial No. 09/500,747 filed 9 February 2000, the contents of which are incorporated herein by reference for this description.

Please replace the paragraph on page 14, commencing at line 27, with the following paragraph:

One useful approach is to modify the KS activity in module 1 which results in the ability to incorporate alternative starter units as well as module 1 extended units. This approach was illustrated in PCT application US/96/11317, incorporated herein by reference, wherein the KS-[I]1 activity was inactivated through mutation. Polyketide synthesis is then initiated by feeding chemically synthesized analogs of module 1 diketide products. The methods of the invention can then be used to provide enhanced amount of extender units.

Please replace the paragraph on page 17, commencing at line 18, with the following paragraph:

As disclosed in [Serial No. 08/989,332 filed 11 December 1997] U.S. Patent No. 6,033,883, incorporated herein by reference, a wide variety of hosts can be used, even though some hosts natively do not contain the appropriate post-translational mechanisms to activate the acyl carrier proteins of the synthases. These hosts can be modified with the appropriate recombinant enzymes to effect these modifications.

Please replace the paragraph on page 22, commencing at line 21, with the following paragraph:

Plasmids pRSG32 (DEBS1+TE) and p132 (a plasmid containing the α and β components of propionyl-CoA carboxylase) were cotransfected into BAP1. Cultures of 10 ml M9 minimal media were grown to mid-log phase levels and concentrated to 1 ml for induction with IPTG and the addition of 0.267 mM ^{14}C-propionate. The samples were then incubated at 22°C for 12-15 hours. The culture supernatant was then extracted with ethyl acetate for analytical TLC. A

product ran with the expected positive control and this same product was undetectable when using either wild type BL-21 (DE3) or removing p132. [t]Thus, the carboxylase forms the correct stereoisomer.

Please replace the paragraph on page 27, commencing at line 27, with the following paragraph:

pCY214 (cm^R) contains the *E. coli* [bira]*birA* (biotin ligase) gene under the ara promoter and is described in Chapman-Smith, *et al., Biochem. J.* (1994) 302:881-887. [This plasmid was obtained as a gift from Dr. Hugo Gramajo.] The PCC protein and *pcc* gene are described in Rodriguez, *et al., Microbiol.* (1999) 145:3109-3119.

In the Claims

1. (Amended) A recombinant *E. coli* [Procaryotic] host cell[s] which [are] is genetically modified for [enhanced] synthesis of [at least one] a polyketide,

wherein said modification comprises

incorporation of [at least one] a propionyl CoA carboxylase (pcc) expression system wherein said pcc expression system produces an enzyme capable of synthesizing 2S-methylmalonyl CoA [expression system for producing a protein that catalyzes the production of starter and/or extender units and/or disabling at least one],

incorporation of at least one expression system for a modular polyketide synthase (PKS), and

incorporation of at least one expression system for a phosphopantetheinyl transferase.